Original Article

Effects of Multi-strain probiotic on Muscle Antioxidant Parameters and Fillet Shelf Life of Rainbow Trout

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How to Cite This Article Amiri, F., Akhondzadeh Basti, A., Taheri Mirghaed, A., Noori, N., & Pourashouri, P. (2025). Effects of Multi-strain probiotic on Muscle Antioxidant Parameters and Fillet Shelf Life of Rainbow Trout. *Iranian Journal of Veterinary Medicine, 19*(1), 81-92. http://dx.doi.org/10.32598/ijvm.19.1.1005400

http://dx.doi.org/10.32598/ijvm.19.1.1005400

Article info:
Received: 30 Jun 2024
Accepted: 25 Jul 2024
Publish: 01 Jan 2025

ABSTRACT

Background: Probiotics are widely used in aquaculture. They can typically improve the growth, immunity, and antioxidant system of fish. However, they can be used to increase fillet shelf life.

Objectives: The present study investigated the effects of dietary supplementation with a commercial probiotic (Bio-Aqua) on growth performance, muscle composition, and shelf life in refrigerated condition.

Methods: Four diets were prepared containing 0 (control=CTR), 0.3 (0.3B), 1 (1B), and 2 (2B) g/kg of the probiotics. Three groups of fish were fed the diets over 12 weeks, and their dorsal muscle chemical composition and antioxidant enzymes were determined. Fillet quality was determined after 0, 4, 8, 12 and 16 days of refrigerated condition.

Results: At the end of the rearing period, there was no significant difference in the growth performance of the fish among the treatment groups. However, the 0.3B group exhibited significantly higher muscle protein content and superoxide dismutase activity. Also, muscle catalase and glutathione peroxidase activities increased dramatically in the 0.3B and 1B treatment groups. Fillet thiobarbituric reactive substances (TBARS), total volatile base nitrogen, total bacterial count, and psychrotrophic bacterial count exhibited elevation over time during refrigerated condition. Dietary probiotics significantly affected the fillet concentration of TBARS, as 0.3B and 1B showed lower TBARS than the CTR and 2B treatment groups. Total bacterial count in probiotic treatments (particularly 0.3B and 1B) was significantly lower than CRT after 8 and 16 days in the refrigerator. There were no interaction effects of dietary probiotics and refrigerated condition time on the fillet total volatile base nitrogen and psychrotrophic bacterial count.

Conclusion: Based on the results, dietary 0.3 or 1 g/kg of Bio-Aqua can improve antioxidant capacity and decrease lipid peroxidation in rainbow trout fillets.

Keywords: Aquaculture, Fillet quality, Peroxidation, Refrigerated condition, Spoilage

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Introduction

Rainbow trout, *oncorhynchus mykiss*, is an important aquaculture candidate with more than 800000 tons of world annual production (Yousefi et al., 2022a). Aquaculture of rainbow trout is an essential sector of the food supply in Iran (Dezfuly et al., 2019), with more than 170000 tons annual production (Yousefi et al., 2022c). Although aquaculture can supply high-quality human food, fish/shellfish meat is very susceptible to perishing due to their relatively high protein content, nitrogenous compounds, and unsaturated fats in muscles (Arulkumar et al., 2017). Refrigeration is one of the methods that can be used to increase the shelf life of fish meat in fish supply centers or for transferring fish from aquaculture centers to markets. Fish storage in refrigerators reduces the rate of enzymatic, chemical, and microbial activities. However, undesirable changes such as oxidation and hydrolysis of fats slowly deteriorate the quality of the products due to the inability of the fridge to minimize fish meat temperature to the necessary level (Pérez-Alonso et al., 2003). Therefore, using methods to improve the quality and increase the shelf life of meat is essential to prevent economic losses and maintain consumer health. Many studies have been conducted on adding preservatives to fish fillets after harvesting (Hussain et al., 2021). However, improving the antioxidant conditions of fish during rearing may help improve the quality of fish fillets in the refrigerator after harvesting.

Probiotics are widely used in aquaculture and can improve fish growth, immunity, and antioxidant systems (Balcázar et al., 2008; Alishahi et al., 2018). For this reason, the use of probiotics in aquaculture increases economic efficiency. The most comprehensive definition of probiotics was provided by Merrifield et al. (2010): "In general, any microbial cell that enters a living organism's body through feed or water in aquaculture and creates beneficial effects for the fish and consumer by improving microbial balance is called a probiotic." Probiotics can create an unfavorable environment for pathogens and control them by various mechanisms such as the production and secretion of inhibitory compounds, competition with pathogenic agents, opportunistic consumption of essential nutrients and attachment sites in the digestive system, as well as stimulating the host's immune system (Balcázar et al., 2008; Wang et al., 2010). Studies have shown that probiotics can improve growth performance, feed utilization, and digestibility of dietary components in fish (Rohani et al., 2021).

Adding probiotics to the fish diet can increase the antioxidant power of fish, as the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase in various fish tissues increases and the amount of thiobarbituric reactive substances (TBARS) decreases (Tovar-Ramírez et al., 2010; Duan et al., 2017; Gobi et al., 2018). However, few studies have focused on the antioxidant effects of probiotics on muscle antioxidant indices and fillet quality during refrigerated condition. For example, the use of the yeast probiotic *Rhodosporidium paludigenum* in the diet of white leg shrimp, *Penaeus vannamei*, led to an increase in the activity of muscle antioxidant enzymes and a decrease in TBARS levels (Yang et al., 2010). Adding the probiotic Bacillus vireti to the freshwater prawn *Macrobrachium rosenbergii* diet improves muscle's antioxidant status after disease occurrence in animals (Vidhya Hindu et al., 2018). Dietary supplementation with *Bacillus subtilis* improves intestinal antioxidant indices and increases physical quality indices of the muscle in crucian carp, *Carassius auratus* (Cao et al., 2019). Adding a probiotic mixture of *Bacillus subtilis*, *Enterococcus faecium*, *Pediococcus acidilactici*, and *Lactobacillus reuteri* to rainbow trout diets increases antioxidant enzyme activity. It decreases TBARS levels in the fish fillet after 5 days of refrigerated condition (Giannenas et al., 2015). Therefore, adding probiotics to fish diets can improve fish's growth and disease resistance and increase fillet quality and shelf life in refrigerated condition, which is important for consumer health. However, further research is needed due to limited studies in this area and the variety of probiotics and fish species (with different muscle compositions) on the market.

In the present study, a commercial probiotic consortium (Bio-Aqua, Biodep Co., Tehran, Iran) composed of 9 bacterial and yeast strains (*P. acidilactici*, *E. faecium*, *B. subtilis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, and *Saccharomyces cerevisiae*) was used for dietary supplementation and administration to rainbow trout. This product has been formulated explicitly for fish and has a 3×10^9 CFU/g cell density. The recommended dose of this product is 0.2-0.3 g/kg, although studies have shown that adding 1-2 g/kg of this product to the diet can increase the growth rate of male and female breeders (Akbari Nargesi et al., 2018; Akbari Nargesi et al., 2019). However, no data shows its benefits on rainbow trout fillet quality in refrigerator. Therefore, the study aims to investigate the effect of adding Bio-Aqua probiotics to rainbow trout diets on antioxidant indices and biochemical quality of fish fillets during refrigerated conditions.

Materials and Methods

Fish diets

This study used a specific rainbow trout feed (Faradaneh Co., Tehran, Iran). Bio-Aqua was a gift from Zist Darman Mahan Co. (Tehran, Iran). To prepare the diets, the probiotic supplement was first mixed with water and then sprayed onto the surface of the pellets. The concentrations used in this study were 0 (control=CTR), 0.3, 1, and 2 g/kg of feed. The amount of 0.3 g/kg was selected based on the manufacturer's recommendation, and the amounts of 1 and 2 g/kg were based on previous studies on rainbow trout (Akbari Nargesi et al., 2018; Akbari Nargesi et al., 2019). After spraying the probiotic onto the feed surface, a gelatin solution was sprayed to prevent the probiotic from being washed away. The feed pellets were used for fish feeding after drying.

Fish culture

In this study, rainbow trout with a mean weight of ~50 g were purchased from a local farm (Aliabad, Golestan Province, Iran). The fish were allowed to acclimatize to the new conditions for 10 days, during which they were fed the control diet and kept in a 2000 L tank with aeration and water flow rate of 5 L/min. Then, 240 fish (61.02±0.13 g) were distributed among twelve 300 L tanks. Each of the three tanks was considered one dietary treatment, and fish were fed daily with the mentioned diets at a rate of 3% biomass (Yousefi et al., 2023a). The rearing tanks were equipped with aeration and constant water flow. Every two weeks, the biomass of each tank was measured to adjust the daily feed amount accordingly. After 12 weeks of culture, the final weight of the fish was recorded, and growth and feed efficiency were calculated based on the Equation 1 (Mirghaed et al., 2023):

1.

$$\text{Weight gain (\%)} = 100\times \frac{Final\ weight\ (g) - Initial\ weight\ (g)}{Initial\ weight\ (g)}$$

$$\text{Specific growth rate (SGR; \%/d)} = 100\times \frac{Ln\ (final\ weigh\ ) - Ln\ (initial\ weight)}{days}$$

$$\text{Feed conversion ratio (FCR)} = \frac{Consumed\ feed\ (g)}{Gained\ biomass\ (g)}$$

The proximate composition and antioxidant indices of fish muscle

At the end of the culture period, three muscle samples were taken from each treatment. For this purpose, a piece of dorsal muscle was cut and immediately frozen in liquid nitrogen (Ahmadifar et al., 2023). These samples were used to investigate the biochemical composition and antioxidant parameters, including the activity of SOD, CAT, and GPx, as well as the amount of TBARS. The biochemical composition of the muscle, including moisture, fat, protein, and ash, was determined according to the Association of Official Analytical Chemists (AOAC) (AOAC, 2023) method. Moisture was measured using an oven at 105°C for 24 h. Fat was measured using ether extraction and a soxhlet apparatus. Protein was determined using the Kjeldahl method by measuring the nitrogen content of the samples. Ash was determined using an electric furnace at 550 °C for 8 h (AOAC, 2023).

The samples were first homogenized in phosphate buffer (pH=7.4) to determine the antioxidant parameters and centrifuged at 4°C (15 min and 13000×g). Then, the upper part of the sample was separated and used to measure the mentioned parameters. The activity of SOD was calculated based on the rate of cytochrome C reduction using a commercial kit from ZellBio Co. (Germany) (Hoseini et al., 2020). CAT activity was measured based on the rate of hydrogen peroxide decomposition. Hydrogen peroxide was used as a substrate, and ammonium molybdate was used as a chromogen and reaction stopper (Goth, 1991). GPx activity was measured based on the rate of glutathione oxidation using a commercial kit from ZellBio Co. (Germany) (Hoseini et al., 2019). TBARS was estimated based on the reaction with thiobarbituric acid at a temperature of 95°C using Oakes et al. (2003) method. In this method, the samples were first deproteinized with trichloroacetic acid. Then, the samples were incubated with a thiobarbituric acid solution in the presence of butylated hydroxytoluene (BHT) for 2 h at a temperature of 95°C to produce a red color. The absorbance of the samples was recorded at a wavelength of 534 nm, and the amount of TBARS was calculated based on the Equation 2:

2. TBARS=Sample absorbance-Blank absorbanc/1560000

Investigation of fillet quality indices during refrigerated storage

Three fillets from each treatment were used to investigate the fillet quality indices. For this purpose, the fillets were placed in a refrigerator, and the total bacterial count, psychrotrophic bacterial count, TVBN and TBARS will be determined on days 0, 4, 8, 12 and 16.

The samples were first homogenized in a 0.85% sodium chloride solution to determine the total bacterial and psychrotrophic bacteria counts. Then, different dilutions of these solutions were prepared and cultured on a nutrient agar medium. The total bacterial count was determined after 48 h of incubation at 37°C, and the psychrotrophic bacteria count was determined after 7 days of incubation at a temperature of 10°C (Ojagh et al., 2010).

The total volatile base nitrogen (TVBN) was determined using Goulas & Kontominas's (2005) method. In this method, magnesium oxide was used to homogenize the samples. Then, the distillation process was performed on the homogenized samples, and the volatile bases were collected in a flask containing boric acid and an indicator. The amount of volatile bases is calculated after titration with sulfuric acid.

Statistical analysis

Normal distribution and homoscedasticity of the data were confirmed by the Shapiro-Wilk and Levene's tests, respectively. Data of growth performance, feed efficiency, survival, muscle antioxidant enzymes' activities, and proximate composition were subjected to one-way ANOVA and Duncan tests. Repeated measure ANOVA and Duncan test analyzed fillet quality parameters during the refrigerated condition. All analyses were performed in SPSS software, version 22 and expressed as Mean±SE.

Results

Growth performance, feed efficiency, and survival of fish within different treatments are presented in Table 1. The treatments had no significant differences in growth performance and feed efficiency. No mortality was observed in various treatments

Proximate composition analysis showed that dietary probiotics did not significantly affect the fish muscle moisture, fat, and ash percentages (Figure 1). On the other hand, the muscle protein percentage increased dramatically in 0.3B, compared to CTR treatment groups.

Table 1. Growth performance, feed efficiency, and survival of rainbow trout in different treatments (n=3)

Variables	Mean±SE				P
	CTR	0.3B	1B	2B	
Initial weight (g)	61.00±0.10^a	61.28±0.36^a	61.78±0.07^a	61.03±0.15^a	0.445
Final weight (g)	258.32±13.17^a	280.63±8.96^a	267.83±10.83^a	267.99±13.88^a	0.636
FCR	1.20±0.07^a	1.04±0.07^a	1.19±0.06^a	1.15±0.09^a	0.458
SGR (%/d)	1.72±0.06^a	1.81±0.03^a	1.76±0.05^a	1.76±0.06^a	0.664
Weight gain (%)	323.54±22.24^a	357.85±12.38^a	140.71±18.34^a	339.06±22.07^a	0.671
Survival (%)	100±0.00^a	100±0.00^a	100±0.00^a	100±0.00^a	1.000

Abbreviations: CTR: Control; FCR: Feed conversion ratio; SGR: Specific growth rate.

Table 2. Effects of probiotics, refrigerated condition time and their interaction on TBARS, TVBN, TBC and PBC of rainbow trout muscle

Parameters	Repeated Measure P		
	Time	Probiotic	Time×Probiotic
TVBN	<0.001(0^a, 4^b, 8^c, 12^c, 16^c)	0.224	0.394
TBARS	<0.001(0^a, 4^{ab}, 8^c, 12^{bcd}, 16^d)	0.012(CTR/2B>0.3B/1B)	0.654
Total bacterial count	<0.001	0.001	<0.001
Psychrotrophic bacterial count	<0.001(0^a, 4^b, 8^c, 12^d, 16^e)	0.817	0.550

Abbreviations: TVBN: Total volatile base nitrogen; TBARS: Fillet thiobarbituric reactive substances; CTR: Control.

Notes: Different superscript letters show significance differences among different sampling times.

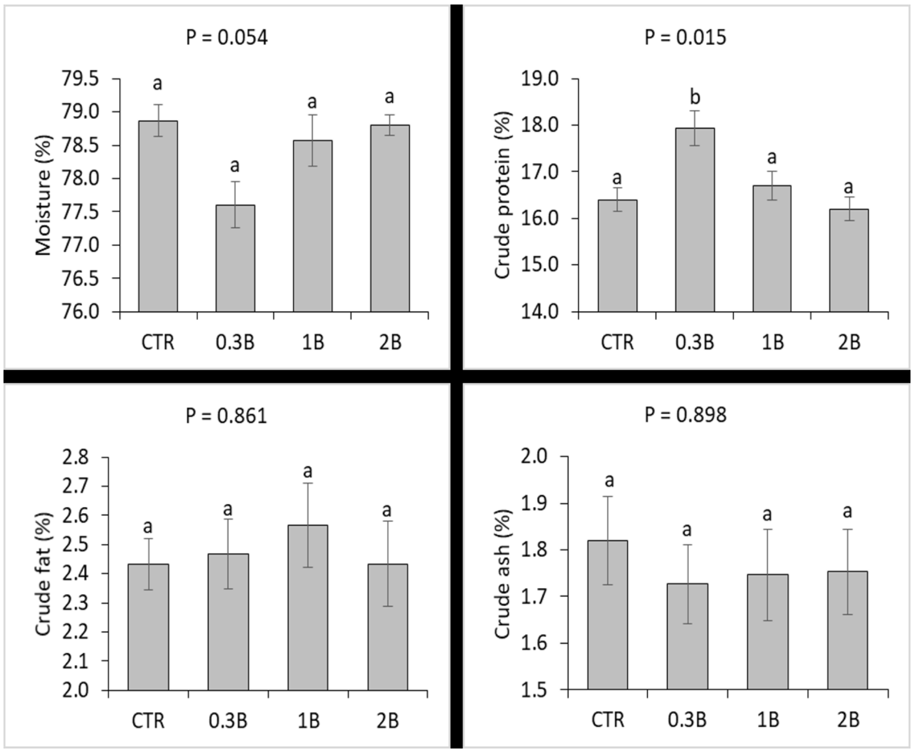

Figure 1. Proximate composition of rainbow trout muscle following 12 weeks feeding on diets supplemented with 0, 0.3, 1, and 2 g/kg probiotics

Note: Different letters above the bars show significant differences among the treatments (n=3).

There was a significant elevation in SOD activity in 0.3B compared to the other treatments (Figure 2). The muscle CAT and GPx activities significantly increased in 0.3B and 1B treatments, compared to CTR and 2B treatments (Figure 2).

Fillet total bacterial count and psychrotrophic bacterial count increased over time during refrigerated condition (Table 2; Figure 3). An interaction effect of dietary probiotics and sampling time was observed in total bacterial count. The probiotic treatments exhibited significantly lower total bacterial count than CTR after 8 and 16 days. The lowest total bacterial counts were observed in 1B and 0.3B treatments after 8 and 16 days, respectively (Figure 3).

Fillet TBARS and TVBN exhibited elevation over time during refrigerated condition (Table 2; Figure 4). Dietary probiotics significantly affected the fillet TBARS concentrations, as 0.3B and 1B showed lower TBARS than CTR and 2B treatments (Figure 3). There were no interaction effects of dietary probiotics and sampling time on the fillet TVBN and TBARS (Figure 4).

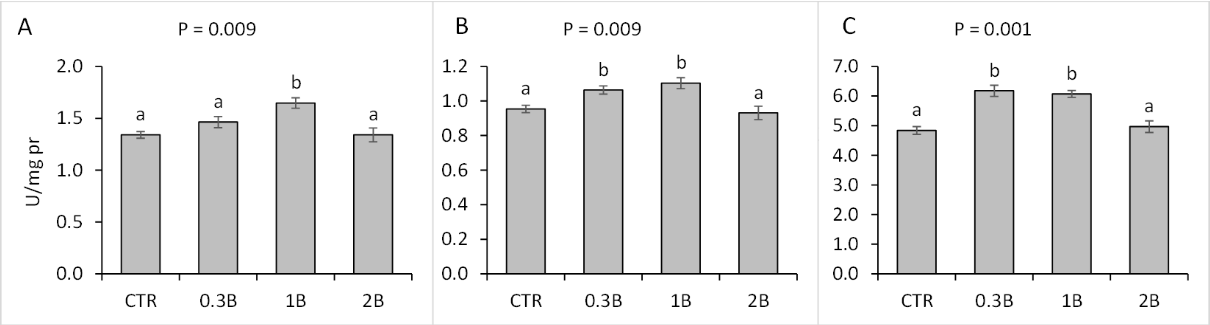

Figure 2. A) SOD, B) CAT and C) GPx activities of rainbow trout muscle in different treatments

Notes: Different letters above the bars show significant differences among the treatments (n=3).

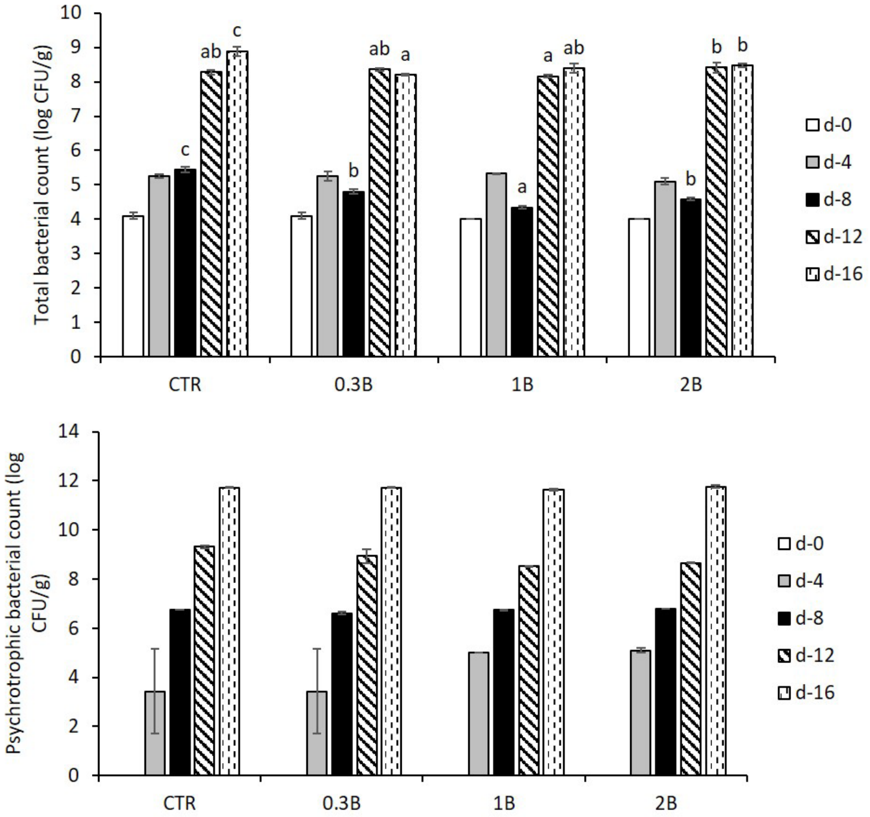

Figure 3. Total bacterial count and psychrotrophic bacterial count of rainbow trout fillet after 16 days keeping in refrigerator

Note: Different letters above the bars show significant differences among the treatments within each time (n=3).

Discussion

Probiotics are used to increase fish growth performance and well-being (Merrifield & Carnevali, 2014; Soltani et al., 2023). However, the growth-promoting effects of probiotics are context-dependent. The present results do not align with Akbari Nargesi et al. (2019) and Akbari Nargesi et al. (2018), who reported growth increase in male and female rainbow trout at 1 and 2 g/kg, respectively. Although several studies have reported enhancement in the growth rate of fish-fed probiotics (El-Haroun et al., 2006; Giannenas et al., 2015; Ramos et al., 2015; Standen et al., 2016), the present results are similar to Shakourian et al. (2021), Nasiripour et al. (2018) and Merrifield et al. (2010) who found no growth enhancement in Siberian sturgeon, *Acipenser baerii*, and rainbow trout, as a result of dietary supplementation with probiotic consortium. Probiotic effects on fish growth are time-dependent. Many studies have shown that a probiotic/probiotic consortium can cause growth-promoting effects over a long time compared to a short time. In the present study, 0.3B treatment exhibited non-significant improvement in fish growth and feed efficiency. Therefore, Bio-Aqua at 0.3 g/kg may increase rainbow trout's growth and feed efficiency over a longer time.

An increase in muscle protein content can be related to various factors. Probiotics have been demonstrated to decrease stress in fish (Gomes et al., 2009), so they may suppress stress-mediated muscle proteolysis, as proposed by Sadoul & Vijayan (2016). On the other hand, probiotics may act against myostatin, a protein that blocks muscle growth (Michelato et al., 2017), as observed in European sea bass, *Dicentrarchus labrax*, administered by dietary *Lactobacillus delbrueckii* (Carnevali et al., 2006). The present results are in line with those obtained in mrigal carp, *Cirrhinus mrigala* (Ullah et al., 2018), largemouth seabass, *Micropterus salmoides* (Wang et al., 2021), and Asian seabass, *Lates calcarifer* (Lin et al., 2017), after dietary administration of probiotic consortiums.

Reactive oxygen species are common products of cell metabolism and are neutralized by antioxidant enzymes (Yousefi et al., 2022b; Yousefi et al., 2023b). Fish rely on their antioxidant system, which includes enzymes

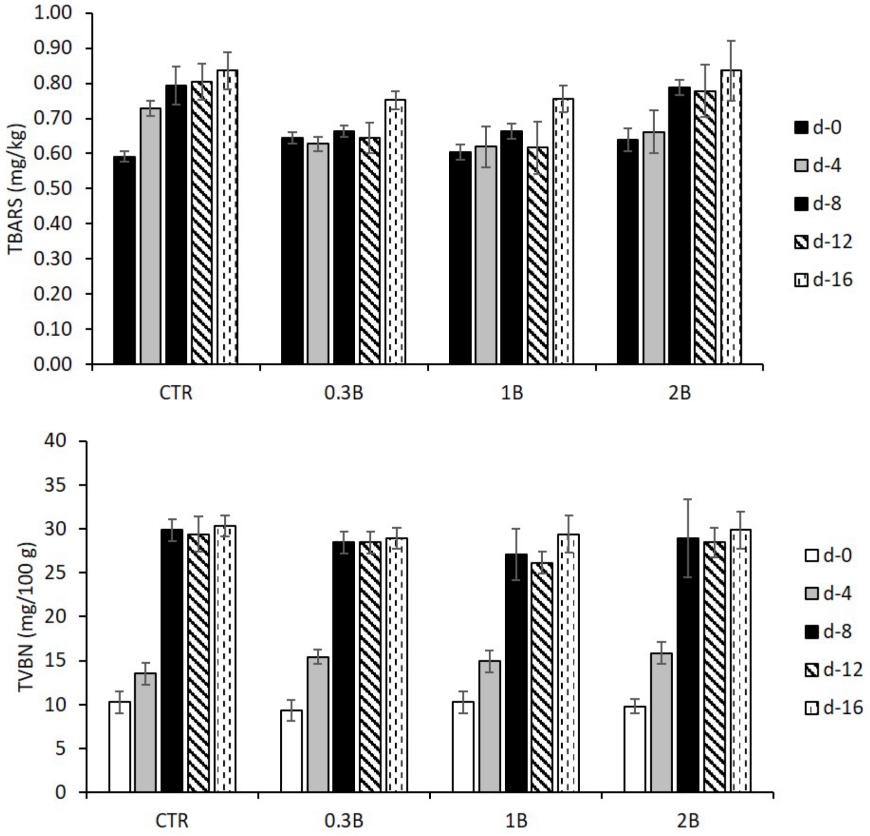

Figure 4. TVBN and fillet TBARS of rainbow trout fillet after 16 days keeping in refrigerator (n=3)

such as SOD, CAT, and GPx (Abbasi et al., 2023; Koohkan et al., 2023). However, if the antioxidant system is overwhelmed, biological materials can be oxidized. Unsaturated fatty acids are particularly vulnerable to oxidation and can lead to the formation of TBARS, which are toxic and can cause further damage to biological materials (Rajabiesterabadi et al., 2020; Hoseini et al., 2022). Probiotics can increase the antioxidant capacity of fish (Hoseinifar et al., 2021), and present results suggest Bio-Aqua improves the activity of SOD, CAT, and GPx in the muscle of rainbow trout. Similar results were obtained by other researchers when rainbow trout (Giannenas et al., 2015), white leg shrimp (Yang et al., 2010), and freshwater prawn (Vidhya Hindu et al., 2018) were treated with dietary probiotics.

Dietary Bio-Aqua supplementation (0.3B and 1B) decreased TBARS content during the refrigerated condition, which aligns with a previous study on rainbow trout (Giannenas et al., 2015). Such benefits can be related to the higher concentration of antioxidants and lower concentration of pro-oxidant compounds in these treatments that protect lipid peroxidation during refrigerated condition when the cellular antioxidant system does not work. Supporting this hypothesis, it has been found that probiotic administration increases the reducing capacity of different fish/shellfish organs (Xie et al., 2019; Chen et al., 2020).

Microbial spoilage is a significant cause of food loss worldwide, accounting for a remarkable portion of annual production losses (Mirsadeghi et al., 2023). Due to microbial activity, fresh and lightly preserved seafood are particularly susceptible to spoilage (Li et al., 2013). To address this issue, monitoring microbial loads in fish fillets during refrigerated condition is inevitable. There is a gradual increase in total bacterial count, psychrotrophic bacterial count, and TVBN over the refrigerated condition period, which is expected (Li et al., 2013; Nowzari et al., 2013; Ramezani et al., 2015). Interestingly, 0.3 and 1 g/kg dietary probiotics decreased total bacterial count after 8 and 16 days of refrigeration storage, indicating the role of Bio-Aqua in decreasing microbial count during refrigeration condition. There is no similar study for comparison, but feeding chicken with diets containing *S. cerevisiae* decreased bacterial load while kept in the refrigerator (Aksu et al., 2005). Applying probiotics in packing fish fillets also showed similar effects (Yasin et al., 2020). On the other hand, probiotics can produce antibacterial compounds with antagonistic effects against harmful microbes (Hamad et al., 2022).

Conclusion

In conclusion, dietary supplementation with Bio-Aqua has no significant effects on the growth performance of rainbow trout. However, 0.3 or 1 g/kg of this probiotic increases muscle protein content and activity of antioxidant enzymes. These concentrations also decrease lipid peroxidation during refrigerated condition storage.

Ethical Considerations

Compliance with ethical guidelines

This study was conducted in accordance of Ethical Guidelines for the Use of Laboratory Animals in Scientific Research" of the University of Tehran, Tehran, Iran.

Funding

The paper was extracted from the PhD dissertation of Faramarz Amiri, approved by the Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Authors' contributions

Conceptualization, supervision and data analysis: Afshin Akhondzadeh Basti, Ali Taheri Mirghaed, Negin Noori, and Parastoo Pourashoori; Methodology: Faramarz Amiri; Data collection: Faramarz Amiri; Investigation: Faramarz Amiri; Writing: All authors.

Conflict of interest

The authors declared no conflict of interest for this article.

Acknowledgments

The authors thanks the staffs of the Inland Waters Aquatics Resources Research Center, Gorgan, Iran for their help in fish rearing and sampling.

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مطالعه پژوهشی

اثرات یک پروبیوتیک تجاری چند سویه بر عملکرد رشد، پارامترهای آنتی اکسیدانی عضلانی و ماندگاری فیله در ماهی قزل آلای رنگین کمان، Oncorhynchus mykiss

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How to Cite This Article Amiri, F., Akhondzadeh Basti, A., Taheri Mirghaed, A., Noori, N., & Pourashouri, P. (2025). Effects of Multi-strain probiotic on Muscle Antioxidant Parameters and Fillet Shelf Life of Rainbow Trout. *Iranian Journal of Veterinary Medicine, 19*(1), 81-92. http://dx.doi.org/10.32598/ijvm.19.1.1005400

doi http://dx.doi.org/10.32598/ijvm.19.1.1005400

چکیده

زمینه مطالعه: پروبیوتیک ها به طور گسترده در آبزی پروری استفاده می شوند و می توانند رشد، ایمنی و سیستم آنتی اکسیدانی ماهی را بهبود بخشند. با این حال، می توان از آنها برای افزایش عمر مفید فیله استفاده کرد.

هدف: مطالعه حاضر به بررسی اثرات مکمل غذایی با یک پروبیوتیک تجاری(Bio-Aqua) بر عملکرد رشد، ترکیب عضلانی و ماندگاری در یخچال پرداخت.

روش کار: چهار جیره حاوی صفر (CTR)، ۰/۳ (0.3B)، ۱ (1B) و ۲ (2B) گرم بر کیلوگرم پروبیوتیک تهیه شد. گروه‌های سه‌گانه ماهی طی یک دوره ۱۲ هفته‌ای با جیره‌های غذایی تغذیه شدند، سپس کیفیت عضلات پشتی پس از ۰، ۴، ۸، ۱۲ و ۱۶ روز در یخچال تعیین شد.

نتایج: در پایان دوره پرورش تفاوت معنی‌داری در عملکرد رشد ماهی در بین تیمارها مشاهده نشد. در تیمار 0.3B پروتئین و فعالیت سوپراکسید دیسموتاز عضله به طور معنی داری افزایش یافت. در حالی که، فعالیت کاتالاز و گلوتاتیون پراکسیداز عضله به طور معنی داری در تیمارهای 0.3B و 1B افزایش یافت. شاخص تیوباربیتوریک اسید، نیتروژن فرار بازی کل، تعداد کل باکتری‌ها و تعداد باکتری‌های سرمادوست عضله با گذشت زمان یخچال گذاری افزایش معنی داری داشتند. پروبیوتیک به طور معنی داری بر شاخص تیوباربیتوریک اسید تأثیر گذاشت، به طوریکه تیمارهای 0.3B و 1B مقادیر کمتری نسبت به تیمارهای CTR و 2B نشان دادند. هیچ اثر متقابل پروبیوتیک و زمان نگهداری در یخچال بر نیتروژن فرار بازی کل ، تعداد کل باکتری ها و تعداد باکتری های سرمادوست عضله وجود نداشت.

نتیجه‌گیری نهایی: بر اساس نتایج، جیره غذایی حاوی ۰/۳ یا ۱ گرم بر کیلوگرم بیو آکوا می‌تواند ظرفیت آنتی‌اکسیدانی را بهبود بخشد و پراکسیداسیون لیپیدی را در عضله قزل‌آلای رنگین‌کمان کاهش دهد.

کلیدواژه‌ها: یخچال گذاری، پراکسیداسیون، فساد، آبزی پروری، کیفیت فیله

تاریخ دریافت: ۱۰ تیر ۱۴۰۳
تاریخ پذیرش: ۲۵ تیر ۱۴۰۳
تاریخ انتشار: ۱۲ دی ۱۴۰۳

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